



ehponline.org

ENVIRONMENTAL HEALTH PERSPECTIVES

Thyroid Hormones in Relation to Lead, Mercury, and Cadmium Exposure in the National Health and Nutrition Examination Survey, 2007–2008

Aimin Chen, Stephani S. Kim, Ethan Chung, Kim N. Dietrich

<http://dx.doi.org/10.1289/ehp.1205239>

Online 16 November 2012



NIEHS

National Institute of
Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Thyroid Hormones in Relation to Lead, Mercury, and Cadmium Exposure in the National Health and Nutrition Examination Survey, 2007–2008

Aimin Chen¹, Stephani S. Kim¹, Ethan Chung¹, Kim N. Dietrich¹

¹Division of Epidemiology and Biostatistics, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

Corresponding Author

Aimin Chen MD PhD

Division of Epidemiology and Biostatistics

Department of Environmental Health

University of Cincinnati College of Medicine

3223 Eden Avenue

Cincinnati, OH 45267-0056, USA

Tel: 513-558-2129

Fax: 513-558-4397

E-mail: aimin.chen@uc.edu

Running Title: Thyroid hormones in relation to heavy metal exposure

Key Words: cadmium, heavy metals, lead, mercury, thyroid hormones

Acknowledgement: This work is partly supported by the Center for Environmental Genetics grant P30ES006096 and grant RC4ES09755 from the National Institute of Environmental Health Sciences.

The authors declare that they have no actual or potential competing financial interests.

Relevant Abbreviations:

CDC – Centers for Disease Control and Prevention

CI – Confidence interval

ETS – Environmental tobacco smoke

FT₃ – Free triiodothyronine

FT₄ – Free thyroxine

GM – Geometric mean

ICP-MS – Inductively Coupled Plasma Mass Spectrometry

IQR – Interquartile range

LOD – Limit of detection

NHANES – National Health and Nutrition Examination Survey

OR – Odds ratio

Tg – Thyroglobulin

TgAb – Thyroglobulin antibody

TPOAb – Thyroid peroxidase antibody

TSH – Thyroid stimulating hormone

TT₃ – Total triiodothyronine

TT₄ – Total thyroxine

Abstract:

Background: Heavy metals, such as lead (Pb), mercury (Hg), and cadmium (Cd), are known toxicants but their associations with the thyroid axis have not been well quantified at background U.S. levels.

Objectives: This study investigated the relationship between thyroid hormones (total and free thyroxine [TT₄ and FT₄], total and free triiodothyronine [TT₃ and FT₃], thyroid stimulating hormone [TSH], and thyroglobulin [Tg]) and Pb, Hg, and Cd concentrations in blood and Cd in urine.

Methods: We separately analyzed a sample of 1109 adolescents (12-19 years) and a sample of 4409 adults from the US National Health and Nutrition Examination Survey (NHANES) 2007-08. We estimated associations after adjustment for age, sex, race, urinary iodine, BMI, and serum cotinine.

Results: The geometric means of blood Pb, total Hg, and Cd were 0.81 µg/dL, 0.47 µg/L, 0.21 µg/L in adolescents and 1.43 µg/dL, 0.96 µg/L, 0.38 µg/L in adults, respectively. The geometric mean of urinary Cd was 0.07 and 0.25 µg/g creatinine in adolescents and adults respectively. No consistent pattern of metal and thyroid hormone associations was observed in adolescents. In adults, blood Hg was inversely related to TT₄, TT₃, and FT₃ and urinary Cd was positively associated with TT₄, TT₃, FT₃, and Tg, but there were no associations with Pb. Associations were relatively weak at an individual level, with about 1-4% change in thyroid hormones per interquartile range increase in Hg or Cd.

Conclusions: The analysis suggests an inverse association between Hg exposure and thyroid hormones and a positive association for Cd in adults.

Introduction

Thyroid hormones (THs) play a critical role in the functions of nervous, reproductive, and cardiovascular systems in both children and adults (Danzi and Klein 2012; Williams 2008; Yazbeck and Sullivan 2012). The hypothalamus-pituitary-thyroid (HPT) axis regulates thyroid function through thyrotropin releasing hormone, thyroid stimulating hormone (TSH), and THs (thyroxine [T_4] and triiodothyronine [T_3]). Circulating T_4 and T_3 are mostly bound to thyroxine binding globulin, transthyretin, and albumin, with less than 1% unbound and biologically active. In peripheral tissues, T_4 is converted to T_3 by type 1 and 2 deiodinases, which in turn binds thyroid receptors α and β and initiates target gene expression (Stathatos 2012). Disruption of TH synthesis, transport, deiodination, and metabolism can result in clinical or subclinical thyroid diseases (Cooper and Biondi 2012). Circulating TSH and THs, even at levels within the reference ranges, are significantly associated with neurodevelopment (Ghassabian et al. 2011; Pop et al. 2003), blood pressure (Asvold et al. 2007), cholesterol, triglycerides, and insulin resistance (Roos et al. 2007).

Environmental chemicals might alter TH levels via several mechanisms, including disruption of iodine transport, thyroid peroxidase, TH binding proteins, hepatic catabolism, deiodinases, and receptor binding (Miller et al. 2009). Studies of human populations have focused primarily on chemicals that are structurally similar to thyroxine, i.e., polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and bisphenol A (BPA), with little attention on heavy metals (Boas et al. 2006; Pearce and Braverman 2009). Lead (Pb), mercury (Hg), and cadmium (Cd) are known environmental toxicants, but only a few studies have examined associations with total and free thyroxine (TT_4 , FT_4), total and free triiodothyronine (TT_3 , FT_3), or

TSH (Dundar et al. 2006; Jin et al. 2006; Lamb et al. 2008; Pearce and Braverman 2009; Robins et al. 1983; Schell et al. 2008).

Lead is known to have adverse neurological, hematological, renal, and gastrointestinal effects (Bellinger 2004; Gurer-Orhan et al. 2004), but associations with THs have been inconsistent (Meeker et al. 2009). Pb exposure (mean 15 µg/dL) was negatively correlated with transthyretin levels in cerebrospinal fluid samples from human patients (Zheng et al. 2001). Previous studies of populations with high exposure to Pb (blood Pb >20 µg/dL) suggested negative associations with circulating T_4 , FT_4 , or T_3 (Lopez et al. 2000; Robins et al. 1983; Singh et al. 2000; Tuppurainen et al. 1988), however, associations were not evident in other study populations (Erfurth et al. 2001; Schumacher et al. 1998; Siegel et al. 1989). Fewer studies have investigated associations of blood Pb levels <10 µg/dL with THs. Dundar and colleagues reported a negative association between blood Pb and FT_4 levels in adolescents with mean blood Pb = 7 µg/dL (Dundar et al. 2006). Recent investigation suggest an inverse association between blood Pb (median 1.5 µg/dL) and TSH levels in men of the couples presenting at infertility clinics (Meeker et al. 2009). Another study in Lakeside Communities of Quebec, Canada found no association between blood Pb (median 3.1 µg/dL) and THs in men, but identified, in females with median blood Pb of 1.7 µg/dL, a positive association with T_3 and an inverse association with TSH (Abdelouahab et al. 2008).

Mercury has adverse effects on a variety of systems that vary with the concentration, length of exposure, and time window of exposure (Tan et al. 2009). Proposed mechanisms of Hg related TH disruption involve selective binding to sulfhydryl (SH)-containing ligands in the thyroid, reduced TSH production, and inhibition of deiodination (Soldin et al. 2008; Tan et al. 2009). FT_3 levels were reduced in association with occupational exposure to Hg vapor among

chloralkali plant workers (Barregard et al. 1994; Ellingsen et al. 2000). Studies of populations with environmental exposure, e.g., from eating fish and from dental amalgams, have had mixed findings (Abdelouahab et al. 2008; Meeker et al. 2009; Schell et al. 2008; Takser et al. 2005). A study in a Canadian lakeside community with exposure levels slightly higher than reported for the US National Health and Nutrition Evaluation Survey (NHANES) [median total Hg 2.25 µg/L in men and 1.50 µg/L in women compared with median total Hg 0.8 µg/L in both men and women for NHANES (Caldwell et al. 2009)] suggested a positive association between Hg and TSH in men only, and no associations with TT₃ and TT₄ (Abdelouahab et al. 2008). Hg was not associated with TSH or FT₄ in 232 Akwesasne Mohawk adolescents with geometric mean Hg = 1 µg/L (Schell et al. 2008).

Cadmium affects renal, skeletal, and respiratory systems and is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (Jarup and Akesson 2009). Cd exposure in animal studies was related to decreased serum TT₄ levels, and interference with deiodination was suggested as a possible mechanism (Hammouda et al. 2008; Mori et al. 2006). A Japanese study comparing residents of the Cd-polluted Kakehashi River basin with residents from a non-polluted area reported that lower FT₄ concentrations in exposed females but higher TT₃ concentrations in both sexes (Nishijo et al. 1994). Studies of neonates and children with environmental exposures have reported inconsistent results (Iijima et al. 2007; Maervoet et al. 2007; Osius et al. 1999). Blood Cd (median 0.2 µg/L) was not associated with TSH in male infertility clinic patients (Meeker et al. 2009).

Many previous studies have had fairly small sample sizes, or have been based on populations with occupational exposures that may not be relevant to the general public. Also, although many studies have measured blood Cd, a good biomarker for recent exposure, urinary

Cd is a better indicator of long-term exposure (Jarup and Akesson 2009). In this report, we analyzed NHANES data from 2007-2008 to estimate associations of Pb, Hg, and Cd with THs in a large U.S. population with background levels of exposure.

Study subjects and methods

We used data from NHANES 2007-2008 (NCHS 2009a) to examine the association between heavy metals and TH in the general population with environmental exposure levels. NHANES is conducted in a nationally representative sample of the US civilian population by the Centers for Disease Control and Prevention (CDC). In 2007-2008, a sample of 10,149 subjects was included in this complex multi-stage stratified cluster survey. Out of these subjects, THs were measured in 6260 subjects aged 12 years or older. We excluded subjects with no blood Pb, Hg, or Cd (n=5), those who had been told by doctor or health professional that they have thyroid problems or were currently taking thyroid medications (n=520) (Belin et al. 2004), and those currently pregnant or taking steroid hormones (estrogen, androgen) that might alter TH or thyroxine binding globulin levels (n=317). The analytical sample for this analysis was 5418, including 1009 adolescents (12-19 years old) and 4409 adults (20-80 years old). After consideration of sampling weights, the analytic sample represented 26,770,162 adolescents and 159,282,838 adults who had no reported thyroid diseases, thyroid medications, pregnancy, and sex steroid medications in the U.S. general population. The analysis was exempt from review at the University of Cincinnati Institutional Review Board (IRB) but each subject provided informed consent to participate in the NHANES study.

Heavy Metals

In the NHANES 2007-2008 cycle, metal assays were conducted in whole blood or urine samples at the Division of Laboratory Sciences, National Center for Environmental Health of the CDC. Whole blood Pb, total Hg, and Cd concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, CDC method No. ITB0001A) with modification from a published method (Nixon et al. 1999), with limits of detection (LOD) 0.25 µg/dL for Pb, 0.33 µg/L for total Hg, and 0.2 µg/L for Cd (NCHS 2009d). Inorganic Hg in whole blood was measured using Flow Injection Mercury System (FIMS) Cold Vapor Atomic Absorption (CVAA), with a LOD of 0.35 µg/L. In the dataset provided by CDC, levels < LOD were imputed as the metal-specific LOD divided by the square root of 2 (Hornung and Reed 1990).

Only 6 participants had blood Pb < LOD. In the U.S. organic Hg accounts for majority of total blood Hg (Mahaffey et al. 2004). Therefore, if the total Hg level was <LOD (n=884), we assumed that organic Hg was equal to the imputed total Hg level (0.2 µg/L). If the total Hg level was > LOD, we calculated organic Hg as the difference between total and inorganic Hg. In this dataset, 4,062 subjects (75%) had inorganic Hg <LOD, thus we did not test associations between inorganic Hg and THs. Blood Cd concentrations were <LOD in 1,282 subjects. In addition to whole blood samples, a 1/3 subset of participants in the NHANES 2007-2008 had urine samples tested for Cd (n=1,767) using ICP-MS. Among them, 106 had urine Cd concentrations <LOD (< 0.042 µg/L). We calculated creatinine-adjusted urinary Cd to account for urine dilution.

Thyroid Hormones

Serum TH and thyroid antibody concentrations were determined in the Department of Laboratory Medicine at the University of Washington, Seattle, WA (NCHS 2009b). Access HYPERSensitive human thyroid-stimulating hormone (hTSH) assay was used to assay TSH.

Competitive binding immunoenzymatic assay was used to determine TT₄, FT₄, TT₃, and FT₃. In addition, NHANES 2007-2008 samples were assayed for thyroglobulin (Tg), thyroglobulin antibody (TgAb), and thyroid peroxidase antibody (TPOAb) using immunoenzymatic assays.

Statistical analyses

In this report, we performed separate analyses for adolescents and adults. In both adolescent (age 12 to 19) and adult (age ≥ 20) samples, we first examined the association between heavy metals and THs using linear regression models. Because both the exposure and outcome variables were not normally distributed, we used natural log transformation to analyze the data. We examined associations of blood Pb, blood total Hg, blood organic Hg, blood Cd, and urinary Cd with TT₄, FT₄, TT₃, FT₃, TSH, and Tg, using separate regression models for each exposure – outcome association. Second, we categorized exposures into quintiles and estimated differences in mean values for the 2nd, 3rd, 4th, and 5th quintiles compared with the first quintile. Third, we examined the proportion of subjects with high concentrations of TgAb (>4 IU/mL) or TPOAb (>9 IU/mL), an indicator of immunologic disturbance of thyroid tissue functions, based on the NHANES laboratory method references (NCHS 2009c). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for metal exposure. Because this analytical sample was thyroid disease and medication free (based on self-report) and the percentage of subjects with clinical and subclinical hyperthyroidism or hypothyroidism was <2% according to NHANES reference values (TSH 0.34-5.6 μ IU/mL) (NCHS 2009c), we did not examine hyperthyroidism or hypothyroidism as dichotomous outcomes. Fourth, we did a subset analysis restricted to women of reproductive age (15-44 years, n=1095) to examine whether metal exposure levels in non-pregnant U.S. women had a discernible association with THs

during reproductive age. Fifth, we summarized significant findings in adolescents and adults by calculating percentage change in THs with an interquartile range (IQR) increase in metal exposure levels. Last, we examined associations between THs and multiple metal exposures by categorizing adult blood Pb, total Hg, Cd by their corresponding medians (1.39 µg/dL, 0.88 µg/L, 0.33 µg/dL) and modeling the 8 possible comparison groups, using the group with all three metals below the median as the reference group.

In the regression models, we adjusted for *a priori* covariates (Caldwell et al. 2009; Hollowell et al. 2002; Muntner et al. 2005; Tellez-Plaza et al. 2012): age (continuous), sex (male, female), race/ethnicity (white, black, Hispanic, and other), natural log transformed creatinine-adjusted urinary iodine (measured by ICP-MS at CDC), body mass index (BMI, age- and sex-specific z-score in adolescent models, original value in adult models), and serum cotinine concentrations (measured by High Performance Liquid Chromatography Tandem Mass Spectrometry at CDC, <1 ng/mL as non-smoking, 1-9.9 ng/mL as environmental tobacco smoke [ETS] exposure, ≥10 ng/mL as active smoking, dummy variables used) (CDC 2009). Since NHANES is a complex multi-stage sampling survey, we used PROC SURVEYREG and PROC SURVEYLOGISTIC in SAS 9.2 (SAS Institute Inc., Cary, NC) to calculate regression parameters and 95% CIs after accounting for sampling weights and survey methods. The significance level was set at $\alpha=0.05$ for two-sided tests.

Results

In the adolescent sample, the mean age was 15.5 years, with 55% male, 60% white, 15% black, and 25% Hispanic and other ethnicity. Twelve percent were exposed to ETS, and 15% were active smokers. The mean BMI z-score was 0.54. The geometric mean (GM) of urinary

iodine was 140 µg/g creatinine. For the adult population, the mean age was 46.4 years, with 55% male, 68% white, 11% black, and 22% Hispanic and other ethnicity. Adults had 28% active smoking percentage, with 5% exposed to ETS. The mean adult BMI was 28.5 kg/m². The GM of adult urinary iodine was 156 µg/g creatinine. The covariates used in adjusted regression models, including age, sex, race and ethnicity, smoking status, BMI, urinary iodine, were associated with metal exposures and THs in most models (detailed data not shown). We also observed increased Pb and Hg levels with age, higher Cd levels in smokers and females, and lower TSH levels in smokers.

Table 1 displays the mean, range and GMs for Pb, Hg, Cd and THs in both adolescents and adults. Adults had higher levels of metal exposures than adolescents with statistically significant differences.

Statistically significant negative associations between blood total Hg and TT₄ and FT₃ were observed in adolescents (Table 2). Blood Cd was positively associated with FT₃ and urinary Cd was positively associated with FT₄. In adults, blood Pb exposure was not associated with any TH levels (Table 3). Both total and organic Hg had significant negative associations with TT₄, TT₃, and FT₃. Blood Cd was positively associated with FT₄ and Tg; urinary Cd was positively associated with TT₄, TT₃, FT₃, and Tg. In the analyses of quintiles of metal exposures and THs in adolescents, the dose-responses were not evident (Supplemental Material, Table S1). However, in adults, the dose-response patterns consistent with the modeling of continuous exposure were evident for total Hg, organic Hg, and urinary Cd (Supplemental Material, Table S2). Higher total blood Hg concentrations were associated with lower TT₄ and TT₃, with the 5th quintile of exposure (≥2.16 µg/dL) showed the strongest associations (Figure 1). Urinary Cd

concentrations were positively associated with TT₄ and TT₃. In contrast, no consistent patterns were found for blood Pb concentrations (Supplemental Material, Table S2).

The percentages of high thyroid antibody levels were slightly higher in adults than adolescents (5.77% vs. 4.79% for TgAb; 8.83% vs. 6.04% for TPOAb, Table 4). However, in neither adolescents nor adults were blood or urinary Pb, Hg, Cd concentrations significantly associated with high TgAb or TPOAb levels.

Subset analyses of continuous exposures among women of reproductive age were generally consistent with associations in the adult population as a whole (see Supplemental Material Table S3). Urinary Cd concentrations were positively related to TT₄, but associations with other THs did not reach statistical significance.

Table 5 gives the estimated percentage difference in TH levels per IQR increase in exposures that were significantly associated with TH when modeled as continuous variables. Overall estimated differences in mean levels were small, at 1-4%. However, we estimated a 12% increase in Tg associated with blood Cd at 0.61 vs. 0.21 µg/L, and an 18% increase in Tg associated with urinary Cd of 0.41 vs. 0.14 µg/g creatinine in adults.

In the three-metal analysis in adults, the negative association between total Hg and TT₄ and TT₃ was evident with and without exposures to Cd or Pb above median levels, and the positive association between blood Cd and Tg was evident for all combinations with exposure to Cd above the median, regardless of exposure to Hg or Pb (see Supplemental Material, Table S4).

Discussion

In adults, Hg exposure was negatively associated with THs, whereas Cd exposure was positively associated with THs and the pre-hormone Tg. TSH was not consistently associated

with Hg or Cd exposure, suggesting that these exposures may not affect pituitary function. In women of reproductive age, the inverse associations between Hg and THs persisted while the associations between Cd and THs were mostly positive but not statistically significant.

The lack of association between blood Pb and THs suggests current exposure levels experienced in the US population do not adversely affect TH synthesis and regulation, though effects of higher environmental exposure levels cannot be ruled out. Occupational Pb exposure has been associated with significant reductions in THs (Robins et al. 1983), and a recent animal study also noted an effect of lead on THs in rats (Wu et al. 2011).

Negative associations observed between Hg and THs are consistent with proposed mechanisms for Hg toxicity in which Hg accumulates in thyroid and reduces iodide uptake at the sodium/iodide symporter by binding to iodide (Nishida et al. 1986), and inhibits TH deiodinase function in peripheral tissues (Soldin et al. 2008; Tan et al. 2009). In the Abdelouahab et al. study (Abdelouahab et al. 2008), TSH was positively associated with hair and blood Hgs, but we did not find an association between TSH and blood Hg in our population. A recent analysis of NHANES 2007-2008 data suggested an increase in the prevalence of TgAb in women with blood Hg >1.8 µg/L compared with ≤0.4 µg/L (Gallagher and Meliker 2012), but that analysis did not exclude subjects that had thyroid disease or were taking medications to treat thyroid disease. We did not evaluate inorganic Hg, which has been associated with higher FT₄/FT₃ ratio XX in two occupational studies (Barregard et al. 1994; Ellingsen et al. 2000). Prior research has suggested that PCBs may influence thyroid hormones, and effects of PCB could therefore confound associations with Hg because both may be consumed in fish (Hagmar 2003). However, we did not have data on PCB exposures.

Studies in experimental animals suggest lower THs in Cd-exposed mice and rats (Gupta and Kar 1997, 1998; Hammouda et al. 2008; Yoshizuka et al. 1991), in contrast with our finding of positive associations between Cd and THs. This discrepancy could be due to species differences or higher exposure doses used in animal studies, although we cannot rule out the possibility that the associations we observed were due to chance or bias. TT₃ was increased in residents of a Cd-polluted region (GM urinary Cd levels 6.6 and 9.2 µg/g creatinine in males and females, respectively) compared with TT₃ levels in residents of a control region (GMs of 2.6 and 4.4 µg/g creatinine, respectively) (Nishijo et al. 1994). In the present study population, GM urinary Cd levels were an order of magnitude lower, but we observed higher THs with increased urinary Cd. Additional research is needed to clarify potential effects of Cd exposure on thyroid function in humans.

The observed associations between Hg and Cd exposures and THs were relatively weak for an individual, with an IQR increase in exposure associated with a 1-4% change in hormone levels. The HPT axis is precisely regulated and it is plausible that low environmental exposures experienced by the US population do not substantially influence individual thyroid profiles. However, exposures at the very high end, e.g., the 5th quintile or higher compared with the 1st quintile, may be associated with a TH level change more than 5%, such as total Hg at ≥ 2.16 µg/L vs. <0.42 µg/L and TT₃ in adults shown in Supplemental Material, Table S2. This may be related to significant health effects in individuals who already have compromised thyroid functions. More research is needed for Hg and Cd exposure at the higher end in specific populations, for example, people who eat a large amount of fish or live close to Cd contaminated areas.

This analysis has limitations related to the cross sectional design of the NHANES. The associations cannot be interpreted as causation, and the results are more relevant to background exposure in the general population. Although we did analyze blood Pb, Hg, and Cd as well as urinary Cd, the latter is only available in a subset of 1/3 sample. The research was only assessing one time point and we lacked longitudinal data. We performed multiple comparisons in the analysis and may encounter the problem of false positive findings. Instead of adopting strict Bonferroni correction, we mainly focused the patterns (relation to more than one TH), dose-response (significance for more than one quintile), and consistency between exposures (total and organic Hg, blood and urinary Cd). In spite of these limitations, we were able to test the associations between metals and FT₄ and FT₃, which were often not measured in prior studies. We stratified the analysis by adolescents and adults, and replicated results in women of reproductive age. We completed the analysis using continuous exposure variable and exposure quintiles, and we summarized percentage change in hormone levels by IQR of exposure.

In the US general adult population, we observed inverse associations between Hg and THs and positive associations between Cd and THs. Research is needed to quantify the associations at higher levels of exposure and to examine potential mechanisms of Hg and Cd thyroid toxicity.

References

- Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, et al. 2008. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). *Environmental Research* 107(3): 380-392.
- Asvold BO, Bjoro T, Nilsen TI, Vatten LJ. 2007. Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population-based study. *Journal of Clinical Endocrinology and Metabolism* 92(3): 841-845.
- Barregard L, Lindstedt G, Schutz A, Sallsten G. 1994. Endocrine function in mercury exposed chloralkali workers. *Occupational and Environmental Medicine* 51(8): 536-540.
- Belin RM, Astor BC, Powe NR, Ladenson PW. 2004. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism* 89(12): 6077-6086.
- Bellinger DC. 2004. Lead. *Pediatrics* 113(4 Suppl): 1016-1022.
- Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. 2006. Environmental chemicals and thyroid function. *Eur J Endocrinol* 154(5): 599-611.
- Caldwell KL, Mortensen ME, Jones RL, Caudill SP, Osterloh JD. 2009. Total blood mercury concentrations in the U.S. population: 1999-2006. *Int J Hyg Environ Health* 212(6): 588-598.
- CDC. 2009. Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals: U.S. Department of Health and Human Services.
- Cooper DS, Biondi B. 2012. Subclinical thyroid disease. *Lancet* 379(9821): 1142-1154.
- Danzi S, Klein I. 2012. Thyroid hormone and the cardiovascular system. *Medical Clinics of North America* 96(2): 257-268.
- Dundar B, Oktem F, Arslan MK, Delibas N, Baykal B, Arslan C, et al. 2006. The effect of long-term low-dose lead exposure on thyroid function in adolescents. *Environ Res* 101(1): 140-145.
- Ellingsen DG, Efskind J, Haug E, Thomassen Y, Martinsen I, Gaarder PI. 2000. Effects of low mercury vapour exposure on the thyroid function in chloralkali workers. *Journal of Applied Toxicology* 20(6): 483-489.

- Erfurth EM, Gerhardsson L, Nilsson A, Rylander L, Schutz A, Skerfving S, et al. 2001. Effects of lead on the endocrine system in lead smelter workers. *Archives of Environmental Health* 56(5): 449-455.
- Gallagher CM, Meliker JR. 2012. Mercury and thyroid autoantibodies in U.S. women, NHANES 2007-2008. *Environment International* 40: 39-43.
- Ghassabian A, Bongers-Schokking JJ, Henrichs J, Jaddoe VW, Visser TJ, Visser W, et al. 2011. Maternal thyroid function during pregnancy and behavioral problems in the offspring: the generation R study. *Pediatric Research* 69(5 Pt 1): 454-459.
- Gupta P, Kar A. 1997. Role of testosterone in ameliorating the cadmium induced inhibition of thyroid function in adult male mouse. *Bulletin of Environmental Contamination and Toxicology* 58(3): 422-428.
- Gupta P, Kar A. 1998. Role of ascorbic acid in cadmium-induced thyroid dysfunction and lipid peroxidation. *Journal of Applied Toxicology* 18(5): 317-320.
- Gurer-Orhan H, Sabir HU, Ozgunes H. 2004. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology* 195(2-3): 147-154.
- Hagmar L. 2003. Polychlorinated biphenyls and thyroid status in humans: a review. *Thyroid* 13(11): 1021-1028.
- Hammouda F, Messaoudi I, El Hani J, Baati T, Said K, Kerkeni A. 2008. Reversal of cadmium-induced thyroid dysfunction by selenium, zinc, or their combination in rat. *Biological Trace Element Research* 126(1-3): 194-203.
- Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. 2002. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism* 87(2): 489-499.
- Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg* 5: 46-51.
- Iijima K, Otake T, Yoshinaga J, Ikegami M, Suzuki E, Naruse H, et al. 2007. Cadmium, lead, and selenium in cord blood and thyroid hormone status of newborns. *Biol Trace Elem Res* 119(1): 10-18.

- Jarup L, Akesson A. 2009. Current status of cadmium as an environmental health problem. *ToxicolApplPharmacol* 238(3): 201-208.
- Jin Y, Liao Y, Lu C, Li G, Yu F, Zhi X, et al. 2006. Health effects in children aged 3-6 years induced by environmental lead exposure. *Ecotoxicology and Environmental Safety* 63(2): 313-317.
- Lamb MR, Janevic T, Liu X, Cooper T, Kline J, Factor-Litvak P. 2008. Environmental lead exposure, maternal thyroid function, and childhood growth. *Environ Res* 106(2): 195-202.
- Lopez CM, Pineiro AE, Nunez N, Avagnina AM, Villaamil EC, Roses OE. 2000. Thyroid hormone changes in males exposed to lead in the Buenos Aires area (Argentina). *Pharmacol Res* 42(6): 599-602.
- Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, et al. 2007. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. *Environ Health Perspect* 115(12): 1780-1786.
- Mahaffey KR, Clickner RP, Bodurow CC. 2004. Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environmental Health Perspectives* 112(5): 562-570.
- Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, et al. 2009. Multiple metals predict prolactin and thyrotropin (TSH) levels in men. *Environ Res* 109(7): 869-873.
- Miller MD, Crofton KM, Rice DC, Zoeller RT. 2009. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environmental Health Perspectives* 117(7): 1033-1041.
- Mori K, Yoshida K, Hoshikawa S, Ito S, Yoshida M, Satoh M, et al. 2006. Effects of perinatal exposure to low doses of cadmium or methylmercury on thyroid hormone metabolism in metallothionein-deficient mouse neonates. *Toxicology* 228(1): 77-84.
- Muntner P, Menke A, DeSalvo KB, Rabito FA, Batuman V. 2005. Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. *Arch Intern Med* 165(18): 2155-2161.
- NCHS. year. (National Center for Health Statistics). National Health and Nutrition Examination Survey 2007-2008. Available: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/nhanes07_08.htm [accessed June 15 2012].

- NCHS. year. (National Center for Health Statistics). National Health and Nutrition Examination Survey 2007-2008 Data Documentation, Codebook, and Frequencies Thyroid Profile (THYROID_E). Available: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/THYROID_E.htm [accessed June 15 2012].
- NCHS. year. (National Center for Health Statistics). National Health and Nutrition Examination Survey 2007-2008 Laboratory Methods. Available: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/lab_methods_07_08.htm [accessed June 15 2012].
- NCHS. year. (National Center for Health Statistics). NHANES 2007 - 2008 Laboratory Files. Available: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/lab07_08.htm [accessed June 15 2012].
- Nishida M, Yamamoto T, Yoshimura Y, Kawada J. 1986. Subacute toxicity of methylmercuric chloride and mercuric chloride on mouse thyroid. *Journal of Pharmacobio-Dynamics* 9(4): 331-338.
- Nishijo M, Nakagawa H, Morikawa Y, Tabata M, Senma M, Miura K, et al. 1994. [A study of thyroid hormone levels of inhabitants of the cadmium-polluted Kakehashi River basin]. *Nihon Eiseigaku Zasshi* 49(2): 598-605.
- Nixon DE, Burritt MF, Moyer TP. 1999. The determination of mercury in whole blood and urine by inductively coupled plasma mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy* 54(8): 1141-1153.
- Osius N, Karmaus W, Kruse H, Witten J. 1999. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ Health Perspect* 107(10): 843-849.
- Pearce EN, Braverman LE. 2009. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab* 23(6): 801-813.
- Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. 2003. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. *Clin Endocrinol (Oxf)* 59(3): 282-288.
- Robins JM, Cullen MR, Connors BB, Kayne RD. 1983. Depressed thyroid indexes associated with occupational exposure to inorganic lead. *Arch Intern Med* 143(2): 220-224.

- Roos A, Bakker SJ, Links TP, Gans RO, Wolffenbuttel BH. 2007. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *Journal of Clinical Endocrinology and Metabolism* 92(2): 491-496.
- Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. 2008. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'-DDE, and other toxicants in Akwesasne Mohawk youth. *Environ Health Perspect* 116(6): 806-813.
- Schumacher C, Brodtkin CA, Alexander B, Cullen M, Rainey PM, van Netten C, et al. 1998. Thyroid function in lead smelter workers: absence of subacute or cumulative effects with moderate lead burdens. *International Archives of Occupational and Environmental Health* 71(7): 453-458.
- Siegel M, Forsyth B, Siegel L, Cullen MR. 1989. The effect of lead on thyroid function in children. *Environ Res* 49(2): 190-196.
- Singh B, Chandran V, Bandhu HK, Mittal BR, Bhattacharya A, Jindal SK, et al. 2000. Impact of lead exposure on pituitary-thyroid axis in humans. *Biometals* 13(2): 187-192.
- Soldin OP, O'Mara DM, Aschner M. 2008. Thyroid hormones and methylmercury toxicity. *Biol Trace Elem Res* 126(1-3): 1-12.
- Stathatos N. 2012. Thyroid physiology. *Medical Clinics of North America* 96(2): 165-173.
- Takser L, Mergler D, Baldwin M, de Grosbois S, Smargiassi A, Lafond J. 2005. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. *Environ Health Perspect* 113(8): 1039-1045.
- Tan SW, Meiller JC, Mahaffey KR. 2009. The endocrine effects of mercury in humans and wildlife. *Critical Reviews in Toxicology* 39(3): 228-269.
- Tellez-Plaza M, Navas-Acien A, Caldwell KL, Menke A, Muntner P, Guallar E. 2012. Reduction in cadmium exposure in the United States population, 1988-2008: the contribution of declining smoking rates. *Environmental Health Perspectives* 120(2): 204-209.
- Tuppurainen M, Wagar G, Kurppa K, Sakari W, Wambugu A, Froseth B, et al. 1988. Thyroid function as assessed by routine laboratory tests of workers with long-term lead exposure. *Scandinavian Journal of Work, Environment and Health* 14(3): 175-180.
- Williams GR. 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. *Journal of Neuroendocrinology* 20(6): 784-794.

- Wu CY, Liu B, Wang HL, Ruan DY. 2011. Levothyroxine rescues the lead-induced hypothyroidism and impairment of long-term potentiation in hippocampal CA1 region of the developmental rats. *Toxicology and Applied Pharmacology* 256(2): 191-197.
- Yazbeck CF, Sullivan SD. 2012. Thyroid disorders during pregnancy. *Medical Clinics of North America* 96(2): 235-256.
- Yoshizuka M, Mori N, Hamasaki K, Tanaka I, Yokoyama M, Hara K, et al. 1991. Cadmium toxicity in the thyroid gland of pregnant rats. *Experimental and Molecular Pathology* 55(1): 97-104.
- Zheng W, Lu YM, Lu GY, Zhao Q, Cheung O, Blaner WS. 2001. Transthyretin, thyroxine, and retinol-binding protein in human cerebrospinal fluid: effect of lead exposure. *Toxicol Sci* 61(1): 107-114.

Table 1. Blood and urinary lead, mercury, cadmium concentrations and thyroid hormone levels in the NHANES 2007-2008

Metal and thyroid hormones	Adolescents					Adults				
	n	Mean	Min ^a	Max ^b	GM ^c	n	Mean	Min ^a	Max ^b	GM ^c
Blood Pb (µg/dL)	1009	0.93	0.18	9.20	0.81	4409	1.75	0.18	33.1	1.43
Total Hg (µg/L)	1009	0.68	0.20	6.98	0.47	4409	1.62	0.20	43.9	0.96
Organic Hg (µg/L)	1005	0.49	0.01	6.73	0.21	4404	1.31	0.01	42.9	0.61
Blood Cd (µg/L)	1009	0.29	0.14	4.70	0.21	4409	0.55	0.14	8.81	0.38
Urinary Cd (µg/g creatinine)	312	0.08	0.01	1.60	0.07	1455	0.34	0.02	4.04	0.25
TT ₄ (µg/dL)	1009	7.46	1.50	18.50	7.34	4409	7.63	2.00	27.6	7.48
FT ₄ (ng/dL)	1009	0.79	0.10	1.50	0.78	4409	0.77	0.30	4.80	0.76
TT ₃ (ng/dL)	1009	130.37	74.00	241.00	128.36	4409	112.50	37.0	632.0	110.4
FT ₃ (pg/mL)	1009	3.64	2.20	6.00	3.61	4409	3.21	1.90	20.70	3.18
TSH (µIU/mL)	1009	1.84	0.01	280.76	1.47	4409	2.01	0.002	80.97	1.60
Tg (ng/mL)	1009	10.83	0.07	353.56	7.64	4409	15.62	0.07	4461.00	9.56

^a: Minimum value; ^b: Maximum value; ^c: Geometric mean

Table 2. Adjusted regression coefficients and 95% confidence intervals of blood and urinary lead, mercury, cadmium in relation to thyroid hormones in adolescents^a

Metal	lnTT ₄	lnFT ₄	lnTT ₃	lnFT ₃	lnTSH	lnTg
ln blood Pb	0.01 (-0.02, 0.04)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.02 (-0.002, 0.04)	-0.05 (-0.18, 0.07)	0.05 (-0.13, 0.24)
ln total Hg	-0.02 (-0.04, -0.001)*	0.005 (-0.01, 0.02)	-0.02 (-0.03, 0.001)	-0.01 (-0.02, -0.003)*	0.02 (-0.04, 0.08)	-0.05 (-0.20, 0.10)
ln organic Hg	-0.01 (-0.03, 0.004)	0.01 (-0.01, 0.02)	-0.01 (-0.02, 0.01)	-0.01 (-0.02, -0.001)*	-0.01 (-0.06, 0.04)	-0.06 (-0.17, 0.04)
ln blood Cd	-0.01 (-0.06, 0.03)	-0.01 (-0.05, 0.04)	-0.01 (-0.03, 0.02)	0.02 (0.0003, 0.03)*	-0.11 (-0.27, 0.04)	0.07 (-0.15, 0.28)
ln urinary Cd	-0.0003 (-0.03, 0.03)	0.04 (0.002, 0.08)*	0.02 (-0.02, 0.06)	0.02 (-0.01, 0.04)	-0.03 (-0.24, 0.17)	0.08 (-0.13, 0.30)

^a: Adjusted for age, sex, race/ethnicity, creatinine adjusted urinary iodine, BMI z-score, and serum cotinine concentration

*: p<0.05

Table 3. Adjusted regression coefficients and 95% confidence intervals of blood and urinary lead, mercury, cadmium in relation to thyroid hormones in adults^a

Metal	lnTT ₄	lnFT ₄	lnTT ₃	lnFT ₃	lnTSH	lnTg
ln blood Pb	-0.01 (-0.02, 0.01)	0.01 (-0.01, 0.02)	-0.0004 (-0.02, 0.02)	0.01 (-0.001, 0.02)	-0.01 (-0.06, 0.04)	0.01 (-0.03, 0.06)
ln total Hg	-0.02 (-0.02, -0.01)*	-0.005 (-0.01, 0.004)	-0.03 (-0.04, -0.01)*	-0.01 (-0.02, -0.01)*	0.004 (-0.02, 0.03)	-0.01 (-0.05, 0.03)
ln organic Hg	-0.01 (-0.02, -0.004)*	-0.004 (-0.01, 0.001)	-0.02 (-0.03, -0.01)*	-0.01 (-0.01, -0.004)*	0.01 (-0.01, 0.03)	0.001 (-0.02, 0.03)
ln blood Cd	0.005 (-0.01, 0.02)	0.01 (0.001, 0.02)*	0.004 (-0.01, 0.02)	0.0002 (-0.01, 0.01)	-0.02 (-0.07, 0.03)	0.10 (0.05, 0.16)*
ln urinary Cd	0.02 (0.001, 0.03)*	0.01 (-0.01, 0.03)	0.03 (0.02, 0.05)*	0.01 (0.002, 0.02)*	-0.04 (-0.09, 0.02)	0.15 (0.23, 0.25)*

^a: Adjusted for age, sex, race/ethnicity, creatinine adjusted urinary iodine, BMI value, and serum cotinine concentration

*: p<0.05

Table 4. Adjusted odds ratios and 95% confidence intervals of high TgAb and high TPOAb per unit change in natural logarithmic metal exposure in adolescents and adults

Metal	Adolescents ^a		Adults ^b	
	High TgAb (>4 IU/mL)	High TPOAb (>9 IU/mL)	High TgAb (>4 IU/mL)	High TPOAb (>9 IU/mL)
%	4.79	6.04	5.77	8.83
ln blood Pb	0.77 (0.37-1.59)	1.16 (0.58-2.33)	1.20 (0.87-1.64)	1.09 (0.82-1.46)
ln total Hg	1.17 (0.64-2.13)	1.15 (0.68-1.95)	0.94 (0.78-1.13)	0.91 (0.80-1.04)
ln organic Hg	1.29 (0.85-1.96)	1.06 (0.66-1.72)	0.95 (0.81-1.11)	0.96 (0.87-1.06)
ln blood Cd	1.18 (0.56-2.51)	1.44 (0.71-2.95)	1.09 (0.74-1.60)	1.14 (0.85-1.51)
ln urinary Cd	0.99 (0.49-2.02)	0.97 (0.41-2.28)	0.84 (0.47-1.48)	1.20 (0.75-1.93)

^a: Adjusted for age, sex, race/ethnicity, creatinine adjusted urinary iodine, BMI z-score, and serum cotinine concentration

^b: Adjusted for age, sex, race/ethnicity, creatinine adjusted urinary iodine, BMI value, and serum cotinine concentration

Table 5. Interpretation of the observed association with an interquartile range (IQR) change in exposure on thyroid hormone concentrations

Population	Metal exposure	IQR (P ₂₅ to P ₇₅) ^a or LOD/sqrt(2) ^b to P ₇₅	lnP ₇₅ -lnP ₂₅ ^a	Association with thyroid hormones	Regression estimate for IQR change and 95% CI	Percentage difference in thyroid hormones and 95% CI for exposure at P ₇₅ compared with P ₂₅ or LOD/sqrt(2) if more than a quarter subjects had exposure <LOD
Adolescents	Total Hg (µg/L)	0.20 ^b -0.82	1.41	↓ TT ₄	-0.03 (-0.05, -0.001)	-2.6 (-5.0, -0.1)
				↓ FT ₃	-0.02 (-0.03, -0.004)	-1.6 (-2.7, -0.4)
	Organic Hg (µg/L)	0.20 ^b -0.53	0.97	↓ FT ₃	-0.01 (-0.02, -0.001)	-0.8 (-1.6, -0.1)
	Blood Cd (µg/L)	0.14 ^b -0.25	0.58	↑ FT ₃	0.01 (0.0001, 0.02)	0.9 (0.01, 1.9)
	Urinary Cd (µg/g creatinine)	0.04-0.11	1.01	↑ FT ₄	0.04 (0.002, 0.08)	4.3 (0.2, 8.5)
Adults	Total Hg (µg/L)	0.49-1.80	1.30	↓ TT ₄	-0.02 (-0.03, -0.008)	-2.0 (-3.0, -0.8)
				↓ TT ₃	-0.03 (-0.05, -0.02)	-3.3 (-5.0, -1.5)
				↓ FT ₃	-0.02 (-0.02, -0.01)	-1.7 (-2.2, -1.2)
	Organic Hg (µg/L)	0.21-1.39	1.89	↓ TT ₄	-0.02 (-0.03, -0.01)	-2.0 (-3.2, -0.8)
				↓ TT ₃	-0.03 (-0.05, -0.01)	-3.2 (-5.2, -1.1)
				↓ FT ₃	-0.01 (-0.02, -0.01)	-1.4 (-2.1, -0.7)

Blood Cd (µg/L)	0.21-0.61	1.07	↑FT ₄	0.01 (0.001, 0.02)	1.0 (0.1, 1.9)
			↑Tg	0.11 (0.06, 0.17)	11.9 (6.0, 18.1)
Urinary Cd (µg/g creatinine)	0.14-0.41	1.07	↑TT ₄	0.02 (0.001, 0.03)	1.9 (0.1, 3.6)
			↑TT ₃	0.04 (0.02, 0.05)	3.6 (1.8, 5.4)
			↑FT ₃	0.01 (0.002, 0.02)	1.0 (0.2, 1.9)
			↑Tg	0.16 (0.07, 0.25)	17.5 (7.7, 28.3)

^a :P₇₅ is the 75th percentile, and P₂₅ is the 25th percentile

^b:Instead of first quartile, the interval starts from <LOD (value replaced with LOD divided by the square root [sqrt] of 2) to reflect more than 25% participants with exposure <LOD

Figure Legend

Figure 1. Estimated thyroid hormone concentrations according to blood total Hg or urinary Cd exposure quintiles in adults, NHANES 2007-2008. A: Natural logarithmic TT₄ by blood total Hg quintiles; B: Natural logarithmic TT₃ by blood total Hg quintiles; C: Natural logarithmic TT₄ by urinary Cd quintiles; D: Natural logarithmic TT₃ by urinary Cd quintiles.

